

Effect of *Torulaspora delbrueckii* Yeast Treatment on Flavanols and Phenolic Acids of Chenin blanc Wines

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The non-*Saccharomyces* yeast *Torulaspora delbrueckii* contributes positively to the sensory properties of wines by affecting aroma and flavour due to changes in alcohols, esters, fatty acids and lactone levels. One of the less-studied aspects of *T. delbrueckii* is its effect on phenolic compounds relating to sensory attributes. An HPLC-DAD technique was used for the quantification of phenolic compounds in Chenin blanc wines made with *S. cerevisiae* and two *T. delbrueckii* yeasts over three vintages. Chemical and sensory data were subjected to ANOVA and PCA. VIN13, M2/1 and VIN13+M2/1 had a positive effect on the phenolic compound concentrations of Chenin blanc wines. Mouthfeel was highest in VIN13+654 wines and astringency highest in VIN13 wines. An association was evident between flavanols, astringency and mouthfeel for the VIN13, M2/1 and VIN13+M2/1 wines. Chenin blanc wines made with M2/1 and VIN13+M2/1 may result in increased phenolic compound concentrations and astringency, whereas 654 and VIN13+654 may result in wines with increased mouthfeel properties.

INTRODUCTION

Chenin blanc grapes have the potential to produce high-quality wines (Marais, 2003) and the choice of yeast for vinification plays an important role in determining the quality of the wine. Yeast choice is a tool that wine producers can use to improve wine quality and change the style of wines. Wine aromas are affected by the levels of esters, alcohols and fatty acids (González-Barreiro *et al.*, 2013; Robinson *et al.*, 2014). Phenolic compounds (flavanols, flavonols, phenolic acids, anthocyanins) are an equally important quality factor in wine, as they are responsible for colour (red wines), astringency, complexity, stability and bitterness (Lesschaev & Noble, 2005; Kennedy, 2008).

There have been different approaches to the quantification of phenolic compounds in wine for chemical profiling, *i.e.* spectroscopic (Andersen & Markham, 2007) or chromatographic techniques (Stefova *et al.*, 2003; Castillo-Muñoz *et al.*, 2010; Moreno *et al.*, 2015; Nelson *et al.*, 2016). Reversed-phase high performance liquid chromatography (RP-HPLC) coupled to a photodiode array detector (DAD) is one of the more commonly used methods for the separation and quantification of phenolic compounds in wine (De Villiers *et al.*, 2004; Liazid *et al.*, 2010; Lorrain *et al.*, 2011; Fanali *et al.*, 2013; Favre *et al.*, 2014; Garaguso & Nardini, 2015; Nelson *et al.*, 2016). The use of RP-HPLC-DAD

constitutes a reliable tool for the quantification of phenolic compounds by allowing maximum absorbance, controlling peak purity and identifying compounds by means of UV-visible spectra and relative retention times.

Saccharomyces cerevisiae yeasts are used in commercial winemaking, but they have been proven to decrease the phenolic concentration of wine through the adsorption of the phenolic compounds onto the yeast cell walls, consequently negatively affecting the quality of wine (Caridi, 2007). The interaction of yeast strains with grape must during fermentation is characterised by yeast metabolites, which are specific to a yeast strain (Romano *et al.*, 2011). Research has shown that the contribution of non-*Saccharomyces* yeasts to wine quality leads to improved sensory attributes, compared to wines made with *S. cerevisiae* yeasts only (Ciani *et al.*, 2010; Ciani & Comitini, 2011; Jolly *et al.*, 2014). *Torulaspora delbrueckii* (anamorph: *Candida colliculosa*) is one of the non-*Saccharomyces* yeasts that has received attention from researchers due to its role in wine aroma and flavour by affecting alcohols, esters, fatty acids and lactone levels (Azzolini *et al.*, 2012; Velázquez *et al.*, 2015). Van Breda *et al.* (2013) investigated the potential of forty-four *Torulaspora delbrueckii* strains in completing fermentation on their own and/or in a combination inoculation with *S. cerevisiae*. Two

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Torulaspora delbrueckii strains, viz. strains M2/1 and 654, showed potential concerning fermentation rate and improved chemical profiles (Lea & Piggott, 1995; Jolly *et al.*, 2014; Van Breda *et al.*, 2014).

This study aimed to identify and quantify phenolic compounds in Chenin blanc wines made with two *T. delbrueckii* yeasts strains (654 and M2/1) and in combination with a commercial *S. cerevisiae* yeast strain (VIN13). The effect of yeast treatment on the sensory attributes of the wines is also reported.

MATERIALS AND METHODS

Torulaspora delbrueckii yeast strains 654 and M2/1 were obtained from the ARC Infruitec-Nietvoorbij's microbiological yeast collection. Commercially active dry *S. cerevisiae* wine yeast (strain VIN13, Anchor Bio-Technologies, Cape Town, South Africa) was used as reference yeast and for co-inoculation.

Small-scale winemaking

Chenin blanc wines were made according to a standardised small-scale (18 L) white winemaking protocol at the Nietvoorbij Research Cellar (Jolly *et al.*, 2003a; Minnaar *et al.*, 2015) over three vintages (2011, 2012, 2013). The grapes were crushed, de-stemmed and immediately pressed (with the skins) to 100 KPa in a small-scale pneumatic press. Sulphur dioxide was adjusted to 50 mg/L, and 0.5 g/hL of Ultrazyme® was added. The juice was allowed to settle overnight at a temperature of 14°C. Clear juice was drawn off into 20 L stainless steel canisters. The inoculum concentration was 30 g/hL for the *S. cerevisiae* active dry yeast (VIN13) and 2×10^6 cfu/mL for the two *T. delbrueckii* yeast strains (654 and M2/1) in pure culture and co-inoculations. The inoculation of *S. cerevisiae* in the combination treatments was performed one hour after the inoculation of *T. delbrueckii* (Jolly *et al.*, 2003b). The yeast treatments used for the production of the Chenin blanc wines therefore were VIN13, 654, M2/1, VIN13+654 and VIN13+M2/1. Fermentation took place at 14°C. Diammonium hydrogen phosphate (50 g/hL) was added to ensure a sufficient supply of nitrogen during fermentation. Free SO₂ was adjusted to 35 mg/L where fermentations were not completed within 32 days. Bentonite (75 g/hL) was added two days after fermentation commenced. The wines were fermented to glucose dryness (< 5 g/L) as tested with a Clinistix™ strip (Bayer, Cape Town). Residual sugar in the bottled wines was confirmed by Winescan® analysis (glucose+fructose). The wines were cold stabilised at 0°C for a minimum of one week, after which they were racked. The small-scale wine fermentations were performed in duplicate for 2011 and in triplicate for the 2012

and 2013 vintages. All wines were filtered through K700 and EK filter sheets and bottled in 750 mL glass bottles. Free SO₂ was adjusted to 35 mg/L at bottling. The wines were stored at 14°C for three months after bottling before they were evaluated sensorially and chemically. Two and three repetitions per treatment were carried out for the first and subsequent two vintages respectively.

Wine phenolic compound analyses

Reversed-phase high performance liquid chromatography (RP-HPLC)

The RP-HPLC determination of phenolic compounds was performed using an Agilent model 1260 HPLC system (Chemetrix, South Africa). The system was equipped with an auto-sampler and a photodiode array detector. A polymer reversed-phase analytical column (PLRP-S 100 Å, 5 µm, 250 x 4.6 mm) with polystyrene divinylbenzene as a stationary phase was used for compound separation (Polymer Laboratories, USA). A gradient mobile phase programme was used for compound elution. Mobile phase A consisted of water/phosphoric acid (985:15 v/v) with a pH of ca. 1.35, and mobile phase B consisted of water/phosphoric acid/acetonitrile (185:15:800 v/v/v) with a pH of ca. 1.25. The following gradient mobile phase programme was used for compound separation: 94% of mobile phase A was used initially at 0 min, 94% to 69% of mobile phase A at 73 min; 69% to 38% of mobile phase A at 78 min; 38% to 94% of mobile phase A at 90 min. The column and the system were equilibrated for 20 minutes after each analysis run time of 90 min to revert to the starting conditions. The flow rate was 1 mL/min. Separation of the compounds was carried out at ca. 25°C. The identification of phenolic compounds was confirmed by their relative retention times based on available phenolic compound reference standards and UV-visible absorption characteristics (Stefova *et al.*, 2003; De Villiers *et al.*, 2011). Wine sample aliquots of 2 mL were filtered through 0.45 µm nylon membrane syringe filters prior to analysis. A 50 µL sample filtrate was injected onto the HPLC column. Replicate samples were analysed on the same day.

Sensory analyses

A panel of twelve experienced wine tasters evaluated the wines. The wine tasting took place over four consecutive days to eliminate the tasting of replicate wines in one session. The wines were presented to each judge in international wine-tasting glasses in a randomised order. Sensory analysis involved the evaluation of mouthfeel, acidity and astringency on a 10 cm unstructured line scale (Tromp & Conradie, 1979).

TABLE 1

Physicochemical parameters measured in Chenin blanc grape musts, indicating average values and standard deviations (2010, 2012 and 2013 vintages).

Parameters	2011	2012	2013
Sugar (°Brix)	21.6 (± 0.32)	20.4 (± 0.22)	20.5 (± 0.45)
Total acidity	7.9 (± 0.34)	7.5 (± 0.25)	8.1 (± 0.55)
pH	3.41 (± 0.23)	3.49 (± 0.32)	3.41 (± 0.37)

Statistical analyses

The sensory and chemical data were analysed using the two-way ANOVA method (SAS Institute, Inc. 1999) to compare treatment means at a confidence level of 95%. Principal component analysis (PCA) was applied to both datasets to determine associations between the variables (sensory attributes and phenolic compounds) and treatments.

RESULTS AND DISCUSSION

Treatment effect on wine physicochemical parameters

Chenin blanc grape juice was analysed prior to inoculation for total soluble solids (°Brix), total acidity and pH (Table 1).

Chenin blanc wines were analysed for volatile acidity, total acidity, alcohol, glycerol, residual sugar and succinic acid (Table 2). There were significant differences between wines made with 654 and VIN13 for volatile acidity, alcohol, residual sugar and succinic acid (2011). VIN13 and 654 wines made during 2012 showed significant differences among volatile acidity, glycerol and residual sugar. In 2013, volatile acidity, glycerol, residual sugar and succinic acid were significantly different between the VIN13 and 654 wines.

Wines made with a combination of VIN13+654 (2011) were significantly different from VIN13 wines for glycerol, residual sugar and succinic acid. VIN13+M2/1 wines (2011) were significantly different from VIN13 wines (2011) for succinic acid. A combination of VIN13+654 wines (2012) were significantly different from VIN13 wines (2012) for residual sugar and succinic acid. VIN13+M2/1 wines (2012) were also significantly different from VIN13 wines (2012) for residual sugar. Wines made with a combination of VIN13+654 (2013) and VIN13+M2/1 (2013) showed no significant differences from the VIN13 wines (2013) in terms of volatile acidity, total acidity, alcohol, glycerol and residual sugar, except for succinic acid, which was significantly different.

Treatment effect on wine phenolic compounds

Two flavanols and four phenolic acids were quantified in the wines (Table 3). The concentrations of phenolic compound, *i.e.* (+)-catechin, (-)-epicatechin, gallic acid, caffeic acid, *p*-coumaric acid and ferulic acid ranged from 5.90 to 16.10 mg/L, 5.64 to 15.99 mg/L, 10.64 to 15.51 mg/L, 2.51 to 13.89 mg/L, 3.95 to 7.07 mg/L and 2.49 to 5.13 mg/L respectively over the three consecutive vintages.

Flavanols

The concentrations of (+)-catechin in wines made with *S. cerevisiae* (VIN13) and *T. delbrueckii* M2/1 strains were higher than in wines made with strain *T. delbrueckii* 654 and VIN13+654 in 2011 and 2012 (Table 3). In 2013, VIN13 wines were lower in (+)-catechin concentrations than in the 654, M2/1 and combination wines. The differences in the concentrations of (+)-catechin in wines made with the two *T. delbrueckii* strains (2011 and 2012) indicate that there are yeast strain differences. The results also showed that yeast strain 654 positively affected (-)-epicatechin concentrations in wines made during 2011, whereas VIN13+654 (2012) and VIN13+M2/1 (2012) positively affected (-)-epicatechin concentrations in comparison to VIN13 wines (2012).

TABLE 2

Oenological parameters measured in Chenin blanc wines (2011 and 2012 vintages).

Oenological parameters	2011						2012					
	Yeast treatment			Yeast treatment			Yeast treatment			Yeast treatment		
	VIN13	654	M2/1	VIN13	654	M2/1	VIN13	654	M2/1	VIN13+654	VIN13+M2/1	VIN13+654+M2/1
² VA (g/L)	0.24 ^b (± 0.02) ¹	0.34 ^a (± 0.01)	0.35 ^a (± 0.01)	0.25 ^b (± 0.01)	0.26 ^b (± 0.02)	0.33 ^b (± 0.02)	0.37 ^a (± 0.09)	0.41 ^a (± 0.06)	0.41 ^a (± 0.06)	0.32 ^b (± 0.03)	0.32 ^b (± 0.03)	0.32 ^b (± 0.03)
³ TA (g/L)	5.79 ^a (± 0.05)	5.68 ^{ba} (± 0.02)	5.66 ^b (± 0.01)	5.81 ^a (± 0.01)	5.71 ^a (± 0.02)	5.41 ^a (± 0.08)	5.41 ^a (± 0.10)	5.39 ^a (± 0.13)	5.39 ^a (± 0.13)	5.37 ^a (± 0.10)	5.39 ^a (± 0.15)	5.39 ^a (± 0.15)
⁴ Alc (%)	13.01 ^a (± 0.04)	11.39 ^b (± 0.38)	11.43 ^b (± 0.41)	12.93 ^a (± 0.42)	12.98 ^a (± 0.03)	12.46 ^a (± 0.06)	12.06 ^{ba} (± 0.61)	11.95 ^b (± 0.45)	11.95 ^b (± 0.45)	12.36 ^a (± 0.09)	12.31 ^a (± 0.02)	12.31 ^a (± 0.02)
⁵ Gly (g/L)	6.85 ^b (± 0.01)	7.02 ^b (± 0.28)	6.69 ^b (± 0.25)	7.46 ^a (± 0.54)	6.85 ^b (± 0.29)	5.80 ^c (± 0.22)	6.30 ^b (± 0.70)	6.93 ^a (± 0.95)	6.93 ^a (± 0.95)	5.84 ^c (± 0.32)	5.73 ^c (± 0.11)	5.73 ^c (± 0.11)
Residual sugar (g/L)	1.71 ^c (± 0.21)	2.68 ^a (± 5.73)	2.34 ^b (± 0.01)	2.73 ^a (± 0.01)	1.93 ^c (± 0.23)	1.82 ^c (± 0.10)	5.23 ^a (± 11.2)	6.57 ^a (± 8.65)	6.57 ^a (± 8.65)	2.07 ^b (± 0.44)	1.91 ^b (± 0.19)	1.91 ^b (± 0.19)
Succinic acid (mg/L)	6.89 ^a (± 0.05)	6.75 ^b (± 0.11)	6.72 ^b (± 0.44)	6.71 ^b (± 0.09)	6.68 ^b (± 0.08)	3.07 ^a (± 0.08)	2.85 ^a (± 0.09)	2.93 ^a (± 0.13)	2.93 ^a (± 0.13)	2.51 ^b (± 0.21)	2.82 ^a (± 0.22)	2.82 ^a (± 0.22)

¹ Standard deviation; ² Volatile acidity; ³ Titratable acidity; ⁴ Alcohol; ⁵ Glycerol

TABLE 2 Continued.
Oenological parameters measured in Chenin blanc wines (2013 vintage).

Oenological parameters	2013				
	Yeast treatments				
	VIN13	654	M2/1	VIN13+654	VIN13+M2/1
2VA (g/L)	0.24 ^b (± 0.11) ¹	0.34 ^a (± 0.05)	0.35 ^a (± 0.02)	0.25 ^b (± 0.01)	0.26 ^b (± 0.03)
3TA (g/L)	6.09 ^a (± 0.01)	5.96 ^a (± 0.01)	5.97 ^a (± 0.02)	6.03 ^a (± 0.04)	6.09 ^a (± 0.07)
4Alc (%)	12.06 ^a (± 0.05)	12.03 ^a (± 0.04)	11.94 ^a (± 0.04)	12.01 ^a (± 0.04)	11.94 ^a (± 0.08)
5Gly (g/L)	7.02 ^b (± 0.09)	7.33 ^a (± 0.22)	7.19 ^a (± 0.07)	6.90 ^b (± 0.11)	7.01 ^b (± 0.15)
Residual sugar (g/L)	1.68 ^b (± 0.23)	2.35 ^a (± 0.48)	2.49 ^a (± 0.51)	1.89 ^b (± 0.39)	1.59 ^b (± 0.33)
Succinic acid (mg/L)	2.52 ^b (± 0.03)	2.11 ^c (± 0.05)	2.51 ^b (± 0.09)	2.08 ^c (± 0.08)	3.35 ^a (± 0.07)

¹ Standard deviation; ² Volatile acidity; ³ Titratable acidity; ⁴ Alcohol; ⁵ Glycerol

¹ Standard deviation; ² Volatile acidity; ³ Titratable acidity; ⁴ Alcohol; ⁵ Glycerol

TABLE 3

Yeast treatment effects on phenolic compounds (mg/L) for Chenin blanc wines made from grapes harvested in the 2011 and 2012 vintages.

Phenolic compounds	2011					2012				
	Yeast treatment					Yeast treatment				
	¹ VIN13	² 654	² M2/1	VIN13+654	VIN13+M2/1	VIN13	654	M2/1	VIN13+654	VIN13+M2/1
Flavanols										
(+)-Catechin	12.05 ^a (± 0.25)	5.90 ^c (± 0.43)	10.00 ^{ab} (± 0.323)	6.91 ^{bc} (± 0.21)	12.05 ^a (± 0.23)	15.18 ^a (± 0.71)	8.44 ^b (± 0.71)	15.34 ^a (± 0.56)	11.79 ^a (± 1.21)	9.42 ^b (± 0.18)
(-)-Epicatechin	5.64 ^a (± 0.17)	6.92 ^a (± 0.76)	5.70 ^a (± 0.38)	6.28 ^a (± 0.22)	6.11 ^a (± 0.62)	9.30 ^{ab} (± 0.46)	9.30 ^{ab} (± 0.39)	8.90 ^b (± 0.53)	9.81 ^b (± 0.19)	10.69 ^a (± 0.12)
Phenolic acids										
Gallic acid	10.64 ^b (± 0.48)	12.13 ^a (± 0.38)	11.47 ^{ab} (± 0.35)	10.74 ^a (± 0.55)	10.71 ^b (± 0.37)	12.27 ^b (± 0.77)	15.51 ^a (± 0.43)	13.57 ^b (± 0.25)	13.45 ^b (± 0.32)	12.76 ^a (± 0.27)
Caffeic acid	2.51 ^a (± 0.01)	3.32 ^a (± 0.05)	3.64 ^a (± 0.08)	3.20 ^a (± 0.09)	4.00 ^a (± 0.11)	11.82 ^a (± 0.22)	11.15 ^a (± 0.45)	12.98 ^a (± 0.44)	12.70 ^a (± 1.41)	13.89 ^a (± 0.98)
<i>p</i> -Coumaric acid	4.48 ^a (± 0.05)	3.95 ^a (± 0.06)	3.97 ^a (± 0.05)	4.52 ^a (± 0.05)	4.52 ^a (± 0.02)	7.07 ^a (± 0.21)	5.70 ^a (± 1.19)	6.09 ^a (± 0.75)	6.99 ^a (± 1.15)	6.90 ^a (± 0.42)
Ferulic acid	2.82 ^a (± 0.41)	2.49 ^a (± 0.43)	2.86 ^a (± 1.37)	2.90 ^a (± 0.34)	2.88 ^a (± 0.45)	2.92 ^c (± 0.62)	2.65 ^c (± 1.15)	3.80 ^a (± 0.18)	3.29 ^{abc} (± 0.55)	3.48 ^{ab} (± 0.12)

* Different superscripts – a, b and c in the same row – indicate significant differences in the content of the compounds among the different treatments according to Fischer's least significant difference test ($p = 0.05$); ¹ VIN13 = *S. cerevisiae*; ² 654, M2/1 = *Torulaspora delbrueckii* strains.

TABLE 3 Continued.
Yeast treatment effects on phenolic compounds (mg/L) for Chenin blanc wines made from grapes harvested in the 2013 vintage.

Phenolic compounds	2013				
	Yeast treatment				
	VIN13	654	M2/1	VIN13+654	VIN13+M2/1
Flavanols					
(+)-Catechin	9.14 ^c (± 1.54)	12.27 ^{ab} (± 2.28)	15.88 ^a (± 1.82)	10.05 ^{bc} (± 2.61)	16.10 ^a (± 2.04)
(-)-Epicatechin	15.99 ^a (± 1.04)	12.87 ^{abc} (± 1.97)	9.46 ^c (± 1.81)	12.87 ^{ab} (± 1.21)	10.91 ^{bc} (± 1.71)
Phenolic acids					
Gallic acid	14.74 ^a (± 0.65)	13.12 ^{ab} (± 0.23)	13.11 ^b (± 0.75)	14.70 ^a (± 0.28)	13.64 ^b (± 0.58)
Caffeic acid	8.62 ^a (± 0.49)	10.37 ^a (± 0.44)	11.88 ^a (± 1.25)	9.22 ^a (± 1.09)	10.40 ^a (± 0.99)
<i>p</i> -Coumaric acid	6.78 ^a (± 0.26)	6.50 ^a (± 0.62)	6.55 ^a (± 0.95)	6.54 ^a (± 0.25)	6.59 ^a (± 0.88)
Ferulic acid	5.13 ^a (± 0.24)	3.13 ^b (± 0.19)	3.64 ^b (± 0.22)	3.58 ^b (± 0.21)	3.40 ^b (± 1.02)

* Different superscripts a, b and c in the same row indicate significant differences in the content of the compounds among the different treatments according to Fischer's least significant difference test ($p = 0.05$); ¹ VIN13 = *S. cerevisiae*; ² 654, M2/1 = *Torulaspora delbrueckii* strains.

The concentration of (+)-catechin in wines made with M2/1 (2013) was notably different (not significantly different) from the 654 wines (2013). Both the M2/1 and 654 wines were significantly different from VIN13 wines for (+)-catechin (2013). Significant differences were evident between the VIN13 and 654 wines (2011, 2012 and 2013) for (+)-catechin. Significant differences were also evident between the VIN13 and VIN13+654 wines (2011) for (+)-catechin. Wines made with M2/1 and VIN13+M2/1 were significantly different in terms of (-)-epicatechin concentrations, and there were also significant differences in concentrations between the VIN13+654 and VIN13+M2/1 wines (2012). During the 2013 vintage, (-)-epicatechin concentrations were significantly different between VIN13 and M2/1 wines, VIN13 and VIN13+M2/1 wines and M2/1 and VIN13+654 wines.

Phenolic acids

Wines made with VIN13 (2011, 2012) proved to be lower in gallic acid than wines made with *T. delbrueckii* strains and the combination wines (Table 3). Gallic acid proved to be highest in VIN13 and VIN13+654 wines made in 2013. Wines made with the two *T. delbrueckii* strains (654 and M2/1) were higher in gallic acid than combination wines (2011 and 2012). *Torulaspora delbrueckii* strain (654 and M2/1) wines made during 2013 were lower in gallic acid than the combination and VIN13 wines.

Caffeic acid was lowest in the VIN13 wines for all three vintages, except for the 654 wines made during 2012, which were lowest in caffeic acid. VIN13+M2/1 wines were highest in caffeic acid for the 2011 and 2012 vintages. In the 2013 vintage, M2/1 wines were highest in caffeic acid concentrations. Wines made with a combination of VIN13+654 (2011) and VIN13+M2/1 (2011) were highest in *p*-coumaric acid concentrations. VIN13 wines proved to be highest in *p*-coumaric acid concentrations for both the 2012 and 2013 vintages. Ferulic acid was highest in wines made with a combination of VIN13+654 in 2011, whereas M2/1 wines and VIN13 wines were highest in ferulic acid concentrations in 2012 and 2013 respectively.

Significant differences were evident between wines made with VIN13 and 654 for gallic acid in both 2011 and 2012. Wines made with VIN13+654 and VIN13+M2/1 (2011 and 2012) showed significant differences between gallic acid concentrations.

Caffeic, *p*-coumaric and ferulic acid concentrations were not significantly different among any of the wines for all five treatments, except for ferulic acid quantified in the VIN13 and 654 wines made during 2012 and the VIN13 wines made during 2013.

Significant differences in ferulic acid were evident between the VIN13 and M2/1 (2012) wines, and between the 654 and M2/1 (2012) wines. VIN13 and 654 wines made during 2013 were significantly different, as were the VIN13 and M2/1 wines. VIN13 and combination wines were also significantly different from each other.

The extraction of phenolics from the grape must correlates with the fermentation rate (sugar consumption) of certain yeast strains (Tables 2 and 3). The higher the sugar consumption, the higher the concentrations of for

certain phenolic compounds in the wine. VIN13 had the highest fermentation rate (sugar consumption), followed by VIN13+M2/1 (2011 and 2012). VIN13+M2/1 had the highest fermentation rate in 2013.

Principal component analysis (Phenolic compounds)

Principal component analysis of the phenolic compound variables was applied to the measurements of each phenolic compound in relation to the total content of the measured variables (Fig. 1). This analysis yielded two principal components, explaining 81.40% of the total variance in the two dimensions (PC1 and PC2), with 61.71% and 19.69% explained by PC1 and PC2 respectively. Association and correlation occurred only in PC1.

The VIN13, 654, M2/1 and combination wines made during 2011 were not associated with any of the measured phenolic compounds (variables), whereas the VIN13 (2012), M2/1 (2012 and 2013), 654 (2013), VIN13+M2/1 (2013) and VIN13+654 (2012) wines were associated with (+)-catechin, caffeic acid, and *p*-coumaric acid. Gallic acid, (-)-epicatechin and ferulic acid were associated with the VIN13 (2013), VIN13+M2/1 (2012), VIN13+654 (2013) and 654 (2012) wines. Yeast treatments that were associated with *p*-coumaric acid (2012, M2/1) were also associated with caffeic acid (2013, M2/1). Wines made with 654 (2013) were associated with phenolic acids, whereas wines made with the same yeast strain in 2013 were associated with phenolic acids and flavanols.

Treatment effect on sensory attributes

Sensory evaluation showed that wines made with 654 and M2/1 (2011 and 2012) scored higher on mouthfeel than wines

made with VIN13 (Table 4), but they were not significantly different. This could be attributed to the higher concentrations of (+)-catechin (M2/1 wines, 2012), (-)-epicatechin (654 wines, 2011), and gallic and caffeic acid concentrations (M2/1 wines, 2011) compared to the VIN13 wines. The high flavanol concentrations suggest that metabolites produced by *T. delbrueckii* yeast strains (654 and M2/1 wines) during fermentation affected the mouthfeel of the wine more than the metabolites produced by the *S. cerevisiae* yeast strain (VIN13). The residual sugar of the 654 (2011 and 2012) and M2/1 (2011 and 2012) wines were higher than that of the VIN13 (2011 and 2012) wines. Glycerol levels were higher in the 654 (2011) wines than in the VIN13 wines (2011). Wines made with the two *T. delbrueckii* yeast strains in 2013 scored lower in mouthfeel than wines made with VIN13 (2013), even though the residual sugar and glycerol levels were higher in the 654 and M2/1 wines (2013) than the VIN13 wines (2013). Wines made with a combination of VIN13+654 scored higher in mouthfeel than the VIN13 wines for both the 2011 and 2012 vintages. In 2013, the VIN13+654 wines scored lower in mouthfeel than the VIN13 wines. A combination of VIN13+M2/1 wines scored lower in mouthfeel (2011 and 2013) than VIN13 wines of the same vintages.

The higher scores for astringency in wines made with *S. cerevisiae* (VIN13) than wines made with the two *T. delbrueckii* strains (654 and M2/1) in all vintages show the effect of yeast strains on the astringency of wine. Wines made with a combination of VIN13+654 and VIN13+M2/1 (2011 and 2012) scored more or less the same as the VIN13 wines for astringency. Wines made with 654 and M2/1, and the combination wines in 2013, scored slightly lower in

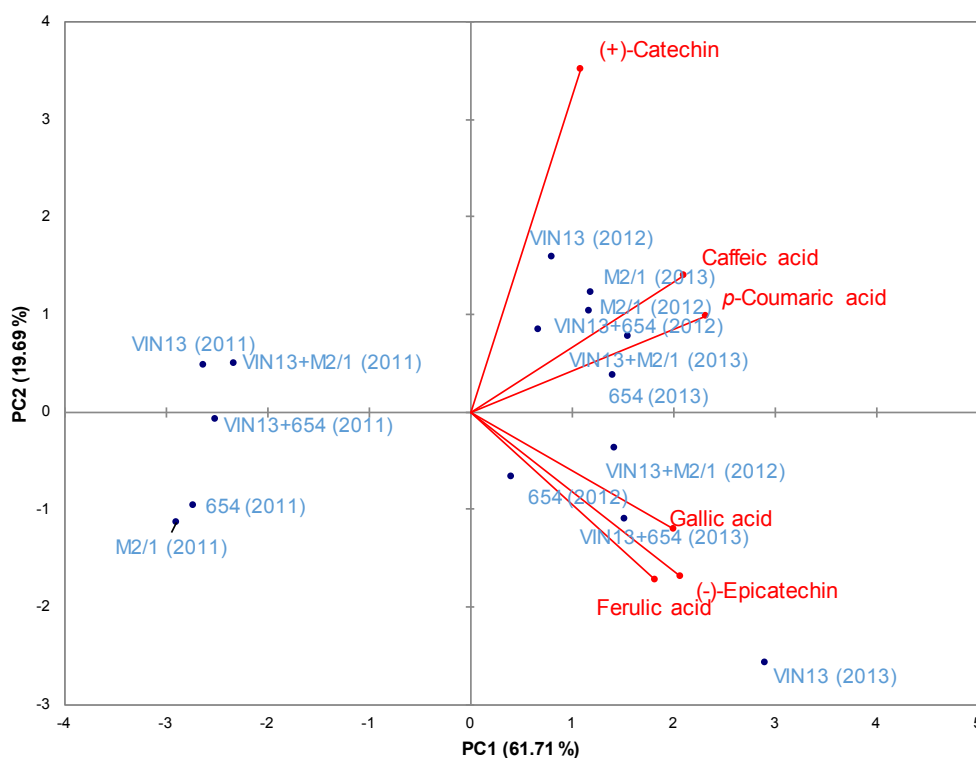


FIGURE 1

Biplot of relative positions and loadings of six phenolic compounds used in PCA for Chenin blanc wines subjected to five treatments, showing differences over three vintages.

TABLE 4

Yeast treatment effect on sensory attributes (%) of Chenin blanc wines made from grapes harvested during 2011, 2012 and 2013 vintages.

Sensory attributes	2011					2012					2013				
	Yeast treatment					Yeast treatment					Yeast treatment				
	¹ VIN13	² 654	² M2/1	VIN13+654	VIN13+M2/1	VIN13	654	M2/1	VIN13+654	VIN13+M2/1	VIN13	654	M2/1	VIN13+654	VIN13+M2/1
Mouthfeel	54.38 ^{a*}	56.23 ^a	55.34 ^a	55.03 ^a	53.57 ^a	50.74 ^a	52.00 ^a	51.89 ^a	53.56 ^a	50.79 ^a	56.57 ^a	53.96 ^{ab}	54.83 ^{ab}	54.93 ^{ab}	50.88 ^b
Astringency	25.53 ^a	14.73 ^b	15.19 ^b	23.92 ^a	24.76 ^a	21.43 ^a	18.76 ^a	19.02 ^a	21.33 ^a	20.46 ^a	25.49 ^a	22.64 ^{ab}	23.86 ^{ab}	20.92 ^b	24.06 ^{ab}
Perceived acidity	45.38 ^{ab}	37.03 ^b	41.46 ^b	52.96 ^a	53.11 ^a	49.61 ^a	47.66 ^a	45.92 ^a	45.56 ^a	46.05 ^a	53.89 ^a	51.90 ^a	52.54 ^a	51.87 ^a	51.54 ^a

* Different superscripts – a and b – on the same line indicate significant differences in the content of the compounds among the different treatments according to Fischer's least significant difference test ($p = 0.05$); ¹ VIN13 = *S. cerevisiae*; ² 654, M2/1 = *Torulaspora delbrueckii* strains.

astringency than the VIN13 wines of the same year.

Perceived acidity was lower in wines made with the two *T. delbrueckii* yeast strains (654 and M2/1) than wines made with the VIN13 strain for all vintages. Wines made with a combination of VIN13+654 (2011) and VIN13+M2/1 (2011) scored higher in perceived acidity than VIN13 wines (2011). Combination wines made during 2012 and 2013 scored lower in perceived acidity than VIN13 wines (2012 and 2013). However, succinic acid can also contribute to perceived acidity (Taillandier *et al.*, 2014; Lleixà *et al.*, 2016). There were significant differences in the concentration of succinic acid among wines made over the three vintages. The low perceived acidity in the 654 and M2/1 wines (2011 and 2012), and the VIN13+654 and VIN13+M2/1 wines (2012 and 2013), can be attributed to the low succinic acid concentrations of the 654 wines (2011, 2012 and 2013), the M2/1 wines (2011 and 2012) and the combination wines (2011 and 2012) compared to the VIN13 wines.

Principal component analysis (sensory attributes)

Principal component analysis for sensory variables was applied to the percentage of each sensory attribute in relation to the total content of the measured variables (. 2). The analysis yielded two principal components (PC1 and PC2) explaining 93.64% of the total variance in the two dimensions (PC1 and PC2), with 60.87% and 32.77% explained by PC1 and PC2 respectively. The association and correlation occurred mostly in PC1.

Wines made with VIN13 (2011, 2012 and 2013), VIN13+654 (2011 and 2013), 654 (2013), VIN13+M2/1 (2011) and M2/1 (2013) were associated with astringency and acidity. Mouthfeel was not associated with any of the wines. VIN13+M2/1 (2012), 654 (2011 and 2012), M2/1 (2011 and 2012), VIN13 (2012) and VIN13+654 (2012) wines were not associated with any of the measured sensory attributes.

CONCLUSIONS

Two flavanols and four phenolic acids were quantified in the Chenin blanc wines. The results show that the VIN13 wines (average values over three vintages) were highest in flavanol concentrations, followed by the M2/1 and VIN13+M2/1 wines. The VIN13 and VIN13+M2/1 wines had the lowest and second lowest residual sugar respectively. Phenolic acid concentrations were highest in the M2/1 wines over the three vintages, followed by the VIN13+M2/1 wines.

Wines made with a combination of VIN13+654 scored highest in mouthfeel. The M2/1 wines were the highest in glycerol concentration, followed by the 654 wines. Glycerol can contribute to mouthfeel. The glycerol concentrations in the M2/1 wines may have influenced the tasters' judgement on mouthfeel. Alcohol concentrations can also affect the mouthfeel properties of wine. The VIN13+654 wines were the highest in alcohol, followed by the VIN13+M2/1 wines. The higher alcohol concentrations of the VIN13+654 and VIN13+M2/1 wines may have influenced the tasters' judgement on mouthfeel.

Wines made with VIN13+M2/1 had the second lowest (out of five) residual sugar levels, whereas VIN13+654

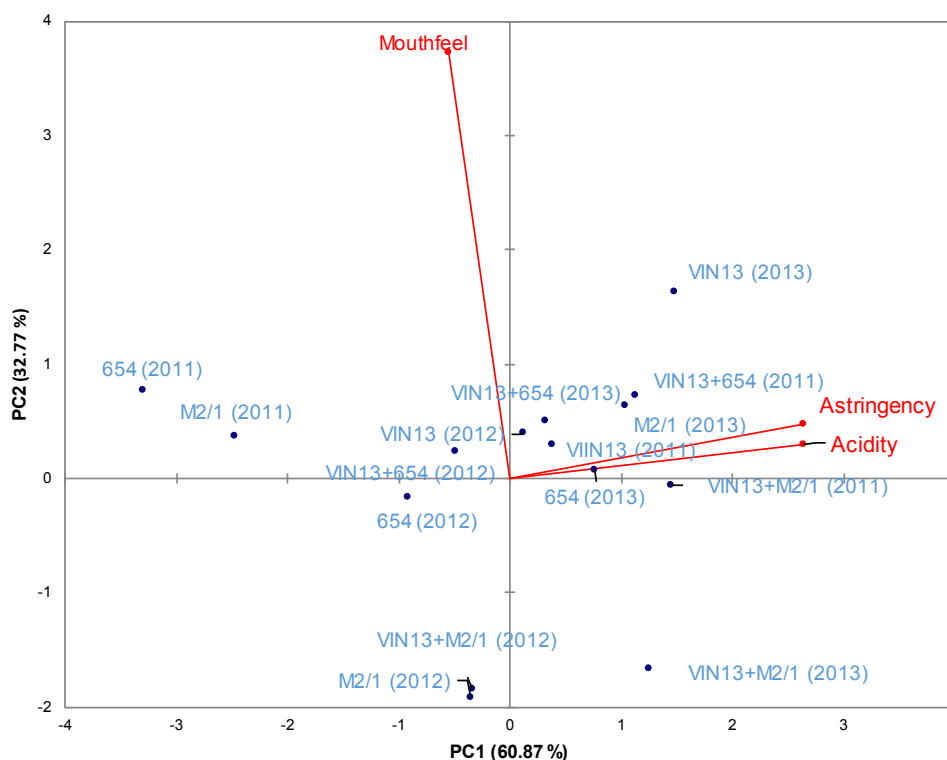


FIGURE 2

Biplot of relative positions and loadings of three sensory attributes used in PCA for Chenin blanc wines subjected to five treatments, showing differences over three vintages.

wines had the third lowest (out of five) residual sugar levels. The residual sugar concentrations of 654 and M2/1 wines may have influenced the tasters' judgement on mouthfeel, whereas this is unlikely in the case of the VIN13+654, VIN13 and VIN13+M2/1 wines.

Wines made with VIN13 were the highest in astringency, followed by the VIN13+M2/1 wines. The least astringent wines were the 654 wines, followed by the M2/1 wines.

Wines made with a combination of VIN13+M2/1 were highest in perceived acidity, followed by the VIN13+654 wines. Succinic acid was highest in the VIN13+M2/1 wines, followed by the VIN13 wines. The high concentrations of succinic acid in the VIN13+M2/1 wines may have contributed to the high perceived acidity of these wines by the tasters. Wines made with VIN13+654 had the lowest succinic acid concentrations, but were second highest in perceived acidity. It therefore is unlikely that succinic acid affected the tasters' judgement on the perceived acidity of the wines.

Considering all the results, it is concluded that glycerol and alcohol may have played a role in the mouthfeel properties of the analysed wines. VIN13+M2/1, M2/1 and VIN13 had a positive effect on the phenolic compound concentrations of Chenin blanc wines, with the VIN13+654 wines scoring highest in mouthfeel, although they were also highest in glycerol and alcohol concentrations. Wines made with VIN13 had the highest astringency, and wines made with VIN13+M2/1 had the highest perceived acidity but also the highest succinic acid concentrations. Considering phenolic compound concentrations, the VIN13, M2/1 and VIN13+M2/1 wines had the highest flavanol concentrations,

while VIN13+654 and VIN13+M2/1 had the highest phenolic acid concentrations but the lowest residual sugar (except for the M2/1 and VIN13+654 wines). Therefore, high sugar consumption is associated with increased flavanol and phenolic acid concentrations. It can be concluded that Chenin blanc wines made with M2/1 and VIN13+M2/1 may result in increased phenolic compound concentrations and astringency, whereas wines made with 654 and VIN13+654 may result in increased mouthfeel properties.

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